The complete mitochondrial genome of *Triuncina daii* (Lepidoptera: Bombycidae) and its phylogenetic implications

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Abstract: The bombycid moth, *Triuncina daii* Xing Wang & Zolotuhin, 2015, plays an important role for analyzing the phylogenetic relationships of the family Bombycidae (Lepidoptera: Bombycoidea). Here we first describe the complete mitochondrial genome (mitogenome) of *T. daii*, which includes thirteen protein-coding genes (PCGs), twenty-two transfer RNA genes, two ribosomal RNA genes and an A+T-rich region, and we find the mitogenome is 15,482 bp in length (GenBank no. KY091643). The genes order and orientation in the *T. daii* mitogenome are similar to other sequenced lepidopteran species. Except for *cox1*, all of the PCGs started with ATN. Twelve PCGs stopped at TAA except for *cox1* which stops at a single T. Thirteen PCGs of available species are used to demonstrate the inner phylogenetic relationships of Bombycoidea. The bombycid species form a monophyletic clade with a bootstrap value of 100% and a posterior probability of 1.00.

Key words: Mitogenome; Protein-coding genes; cytochrome *c* oxidase subunit I (*cox1*); Nucleotide composition

戴氏褚蚕蛾线粒体基因组全序列测定及分析（鳞翅目：蚕蛾科）

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摘要：戴氏褚蚕蛾 *Triuncina daii* Xing Wang & Zolotuhin, 2015 在蚕蛾科（鳞翅目：蚕蛾总科）昆虫系统发育关系分析中扮演着重要角色。本实验对戴氏褚蚕蛾的全线粒体基因组（mitogenome）进行了描述，其全长为 15,482 bp（GenBank 登录号为 KY091643），包含 13 个蛋白质编码基因（protein coding genes, PCGs），22 个 tRNA、2 个 rRNA 基因及 1 个 A + T 富集区，基因的排列顺序和方向与其他已测定的鳞翅目昆虫典型线粒体基因组一致。除 *cox1* 基因外，所有 PCGs 均以 ATN 为起始密码子、TAN 为终止密码子。我们选用所有已知的蚕蛾总科 Bombycoidea 昆虫线粒体基因组中的 13 个 PCGs 的核酸序列作为数据集分析其系统发育关系。最大似然法和贝叶斯法分析结果显示蚕蛾科昆虫形成一个单系群，自展检验值为 100%，贝叶斯后验概率为 1.00。

关键词：线粒体基因组；蛋白质编码基因；细胞色素 c 氧化酶亚基 I (*cox1*)；核苷酸组成

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Introduction

Members of the bombycid genus *Triuncina* are distributed in South China, North Vietnam, and Thailand, and comprise of five species. The knowledge about the host plants and ecology of members of this genus is poorly known, and the molecular information of genus *Triuncina* has not yet been reported. The Chinese species, *Triuncina daii* Xing Wang & Zolotuhin, 2015, has been described from Nanling National NR, Guangdong (Wang *et al.* 2015). Here we describe the mitochondrial and genetic characteristics of this species as an example from this genus toward improving our knowledge of this genus and clarifying the phylogenic relationship of *T. daii*.

In Lepidoptera, mitochondrial DNA (mtDNA) typically encode thirteen protein-coding genes (PCGs), twenty-two tRNA genes and two rRNA genes (Wolstenholme 1992; Boore 1999), and a non-coding region (A+T-rich region) which generally represents the gene replication sites and the start site for group transcription (Wolstenholme 1992). The mitogenome, including maternal inheritance and lack of genetic recombination, provides an appropriate source of data for phylogenetic classification and identification. Furthermore, the mitogenome also provides information beyond the mitochondrial coding genes, for instance: evolutionary rates, codon usages, evolutionary patterns, the secondary structures of transfer RNA (tRNA) and ribosomal RNA (rRNA) genes and the features of the long non-coding region (A+T rich region).

As a well-known lepidopteran taxon, the family Bombycidae *s. lat.* contains about 40 genera and roughly 350 species (Lemaire & Minet 1999). The quantity of molecular information for bombycids is still at a low level with only five species where the complete mitochondrial genome has been sequenced: *Bombyx mandarina* (Moore) (NC_003395), *Bombyx huttoni* Westwood (NC_026518), *Bombyx mori* (Linnaeus) (NC_002355), *Rondotia menciana* Moore (NC_021962) (Yukuhiro *et al.* 2002; Peng *et al.* 2015; Kong & Yang 2015) and *Andraca theae* (Matsumura) (KX365419) (Gu *et al.* 2016).

In this study, the mitochondrial genome of *T. daii* is sequenced for the first time and compared with other bombycoid species. The phylogenetic position of *Triuncina* is discussed, based on the mitochondrial genome, to determine a more far-reaching phylogeny among the Bombycidae.

Material and methods

Samples of *T. daii* were collected from Nanling National NR, Ruyuan County, Guangdong Province in China, and stored at 4°C in a refrigerator. Identification of this species follows Wang *et al.* (2015). Larvae were collected and extraction performed to obtain total genomic DNA using the Wizard Genomic DNA Purification Kit (Promega, Beijing, China) according to the manufacturer's instructions.

The complete mtDNA of *T. daii* was amplified by 25.0 μL reactions which contained 0.5 μL rTaq (TaKaRa Co., Dalian, China), 1.0 μL DNA, 2.5 μL 10×rTaq buffer (Mg^{2+}free), 2.0 μL dNTPs, 17.0 μL ddH_2O, and 1.0 μL of each primer. PCR conditions were performed using the following parameters: 1 cycle at 94°C for 1 min, 92°C for 10s, 46°C–60°C for 90s, 35 cycles at 60°C for 1–3 min, and a subsequent 6 min final extension at 72°C. PCR products were detected by 1% agarose gel electrophoresis. The amplified fragments were sequenced directly by the appropriate primers using a commercial kit (Bioteke, Beijing, China).

The program Geneious version 8.1.2 (Kearse *et al*. 2012) were used to assemble and proof-read the fragments of the *T. daii* mitogenome. Following completion of the sequencing of the mitogenome, it was then annotated manually and automatically. The method of written annotation follows Cameron & Whiting (2008) and the online-program MITOS (Bernt *et al*. 2013) (http://mitos.bioinf.uni-leipzaig.de) was used to complete the automated annotation. Protein-coding gene boundaries were verified by the ORF finder (http://www.ncbi.nlm.nih.gov/orf/gorf.html), and the start and termination codons of PCGs were determined by MEGA version 6.0 (Tamura *et al*. 2013). The tRNA genes were identified using the tRNAscan-SE program (http://lowelab.ucsc.edu/tRNAscan-SE/) (Lowe & Eddy 1997). Unidentified tRNAs were compared with sequences from other species. The two rRNA subunit coding genes (*rrnL* and *rrnS*) were identified by NCBI Internet BLAST search. Geneious version 8.1.2 was using for calculating the A+T content and codon usage (Kearse *et al*. 2012). The skew of the compositions were determined using the formulas as followed: AT skew = [A - T] / [A + T]; GC skew = [G - C] / [G + C] (Junqueira *et al*. 2004).

Table 1. Source and information for phylogenetic analysis

Species	**Family**	**Subfamily**	**Accession number**	**References**
Actias artemis	Saturniidae	Saturniinae	KF927042	Park *et al*. (2016)
Actias selene	Saturniidae	Saturniinae	NC_018133	Liu *et al*. (2012)
Andraca theae	Bombycidae	Bombycinae	KX365419	Gu *et al*. (2016)
Antheraea frithi	Saturniidae	Saturniinae	NC_027071	Unpublished
Antheraea pernyi	Saturniidae	Saturniinae	NC_004622	Liu *et al*. (2008)
Antheraea yamamai	Saturniidae	Saturniinae	NC_012739	Kim *et al*. (2009)
Apatelopteryx phenax	Lasiocampidae	-	KJ508055	Timmermans *et al*. (2014)
Attacus atlas	Saturniidae	Saturniinae	NC_021770	Chen *et al*. (2014)
Bombyx huttoni	Bombycidae	Bombycinae	NC_026518	Peng *et al*. (2015)
Bombyx mandarina	Bombycidae	Bombycinae	NC_003395	Yukuhiro *et al*. (2002)
Bombyx mori	Bombycidae	Bombycinae	NC_002355	unpublished
Dendrolimus punctatus	Lasiocampidae	-	NC_027156	unpublished
Dendrolimus spectabilis	Lasiocampidae	-	NC_025763	Tang *et al*. (2014)
Dendrolimus tabulaeformis	Lasiocampidae	-	NC_027157	Unpublished
Eriogyna pyretorum	Saturniidae	Saturniinae	NC_012727	Jiang *et al*. (2009)
Euthrix laeta	Lasiocampidae	-	NC_031507	Unpublished
Manduca sexta	Sphingidae	Sphinginae	NC_010266	Cameron and Whiting (2008)
Notonagemia analis	Sphingidae	Sphinginae	KU934302	Kim *et al*. (2016)
Rondotia menciana	Bombycida	Bombycinae	NC_021962	Kong and Yang (2015)
Samia canningi	Saturniidae	Saturniinae	NC_024270	Shantibala *et al*. (2016)
Samia Cynthia	Saturniidae	Saturniinae	KC812618	Sima *et al*. (2013)
Samia ricini	Saturniidae	Saturniinae	NC_017869	Kim *et al*. (2012)
Saturnia boisduvalii	Saturniidae	Saturniinae	NC_010613	Hong *et al*. (2008)
Sphinx morio	Sphingidae	Sphinginae	NC_020780	Kim *et al*. (2013)
Triuncina daii	Bombycida	Bombycinae	KY091643	This study

The 26 mitogenomes of species were downloaded from NCBI (Table 1) and include 24 bombycoid species considered as ingroups and 2 geometrid species, *Phthonandria atrilineata* and *Biston panterinaria* (Kong & Yang 2015), serving as outgroups. The phylogenetic tree was constructed using the nucleotides of 13 protein-coding genes which excluded the stop codon and were combined. The comparative analysis and spread correction were performed to sequence by the software Mega 6.0 (Tamura *et al*. 2013). Gblock 0.91b with default settings was used to filtrate the conserved regions of the putative amino acids (Castresana 2000).

The 13 individual PCG sequences were conducted by the Akaike Information Criterion (AIC) with the jModelTest 2.1.5 (Ronquist & Huelsenbeck 2003). The BI analyses was performed via MrBayes v3.2.3 (Darriba *et al*. 2012), and were run for 1,000,000 generations with trees being sampled every 1000 generations, with four chains and a burn-in step for the first 10,000 generations. The ML method used the program raxmlGUL 1.5 (https://sourceforge.net/projects/raxmlgui/) (Silvestro & Michalak 2012) and the analysis setting chosen was "ML+rapid bootstrap" and the "number of bootstrap replicates" was 1,000.

Results

The complete mitochondrial genome of *T. daii* is a 15,482-bp closed-circular molecule, encoding an entire set of 37 genes which include 13 PCGs (*cox1-3*, *nad1-6*, *nad4l*, *atp6*, *atp8* and *cytb*), 22 tRNA genes, 2 rRNA genes, and a putative A+T-rich region (Table 2). The distribution of genes in the mitochondrial genome is conserved as in the other Lepidopteran mitochondrial genomes (Fig. 1). 23 of the genes are transcribed on the major strand (J-strand), and the other 14 are transcribed on the minor strand (N-strand).

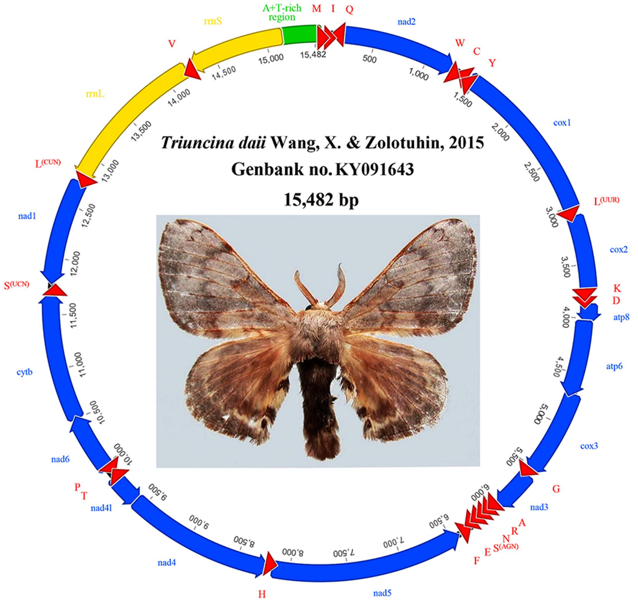

Figure 1. Circular map of the *Triuncina daii* mitochondrial genome. Gene names are annotated using standard abbreviations; single letters are indicated based on the IUPAC-IUB abbreviation of the corresponding amino acid.

The intergenic spacer sequence was 226 bp in total and included 16 non-coding regions which range in size from 1 to 58 bp. There are 4 spacer spans of more than 20 bp which are located between *trnQ- nad2* (44bp), *nad3- trnA* (20bp), *trnF- nad5* (58bp), and *trnS* (UCN)-*nad1* (31bp). The overlapping nucleotide fragments are scattered in 6 places, located between *trnQ* and *nad2* (-3bp), *trnW* and *trnC* (-8bp), *atp8* and *atp6* (-7bp), *trnE* and *trnF* (-2bp), *nad5* and *trnH* (-3bp), and *trnL* (CUN) and *rrnL* (-25bp).

The overall nucleotide composition of *T. daii* mtDNA is as follows: 40.89% A, 11.27% T, 7.18% C, and 40.66% G, with a total A+T content that is significantly biased (81.54%). The positive and negative skew are obviously distinct with an AT skew value of 0.003 and GC skew value of -0.221.

Table 2. Annotation and gene organization of the *Triuncina daii* mitogenome

Gene	Position (bp)	Length (bp)	Direction	Intergenic nucleotides (IGN)	Anti-codons	Start/stop codons	AT%
trnM	1-67	67	J		CAT		77.61%
trnI	68-132	65	J	0	GAT		76.92%
trnQ	130-198	69	N	-3	TTG		82.61%
nad2	243-1265	1023	J	44		ATA/TAA	86.51%
trnW	1281-1350	70	J	15	TCA		84.29%
trnC	1343-1408	66	N	-8	GCA		81.82%
trnY	1409-1473	65	N	5	GTA		80.00%
cox1	1479-3009	1531	J	0		CGA/T	72.63%
trnL(UUR)	3010-3076	67	J	0	TAA		76.12%
cox2	3077-3758	682	J	0		ATG/T	77.86%
trnK	3759-3829	71	J	0	CTT		70.42%
trnD	3830-3896	67	J	0	GTC		88.06%
atp8	3897-4058	162	J	0		ATT/TAA	91.82%
atp6	4052-4729	678	J	-7		ATG/TAA	79.50%
cox3	4737-5525	789	J	7		ATG/TAA	74.90%
trnG	5528-5594	67	J	2	TCC		89.55%
nad3	5595-5948	354	J	0		ATT/TAA	82.20%
trnA	5969-6034	66	J	20	TGC		81.82%
trnR	6036-6099	64	J	1	TCG		79.69%
trnN	6100-6164	65	J	0	GTT		78.46%
trnS(AGN)	6165-6231	67	J	0	GCT		82.09%
trnE	6237-6301	65	J	5	TTC		92.31%
trnF	6300-6367	68	N	-2	GAA		83.82%
nad5	6426-8168	1743	N	58		ATA/TAA	82.85%
trnH	8166-8231	66	N	-3	GTG		83.33%
nad4	8235-9575	1341	N	3		ATG/TAA	81.95%
nad4l	9578-9868	291	N	2		ATG/TAA	85.07%
trnT	9873-9938	66	J	4	TGT		83.33%
trnP	9939-10004	66	N	0	TGG		81.82%
nad6	10007-10534	528	J	2		ATA/TAA	87.50%
Cytb	10548-11702	1155	J	13		ATG/TAA	77.23%

Continued Table 2.

Gene	Position (bp)	Length (bp)	Direction	Intergenic nucleotides (IGN)	Anti-codons	Start/stop codons	AT%
trnS(UCN)	11716-11781	66	J	13	TGA		80.30%
nad1	11813-12751	939	N	31		ATG/TAA	79.34%
trnL(CUN)	12753-12822	70	N	1	TAG		78.57%
rrnL	12798-14189	1392	N	-25			84.48%
trnV	14190-14258	69	N	0	TAC		82.61%
rrnS	14259-15121	863	N	0			86.21%
A+T-rich region	15122-15482	361	-	0			94.18%

Regarding the PCGs, the major strand included *nad2*, *cox1*, *cox2*, *atp8*, *atp6*, *cox3*, *nad3*, *nad6*, and *cytb*, while the minor strand harbored *nad5, nad4, nad4l, and nad1*. Most of the PCGs had the start codon ATN except for *cox1*, where the start codon was CGA. Seven PCGs started at ATG: *cox2*, *atp6*, *cox3*, *nad4*, *nad4l*, *cytb* and *nad1*. The gene *nad2* and *nad5* started with ATA, while, *atp8* and *nad3* started with ATT. Both *cox1* and *cox2* ended with a single T, and the other eleven PCGs stopped at TAA.

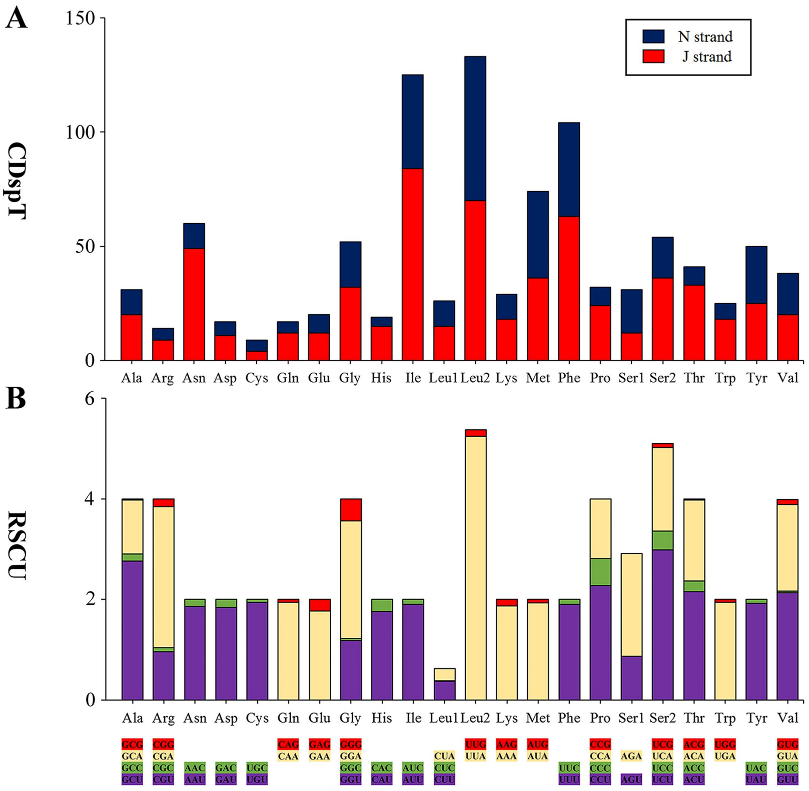

Figure 2. Codon usage in the *Triuncina daii* mitogenome. (A) CDspT (codons per thousand) indicates the codons used in coding amino acids per thousand codons. Codon families are given on the x-axis. (B) RSCU.

The sequences of protein-coding genes contained a total of 11,213bp codons excluding the start and termination codons. The content of A+T was 80.10% which is far higher than G+C. The Relative Synonymous Codon Usage (RSCU) values in the PCGs of the *T. daii* mitogenome are shown in Table 3 and Fig. 2.

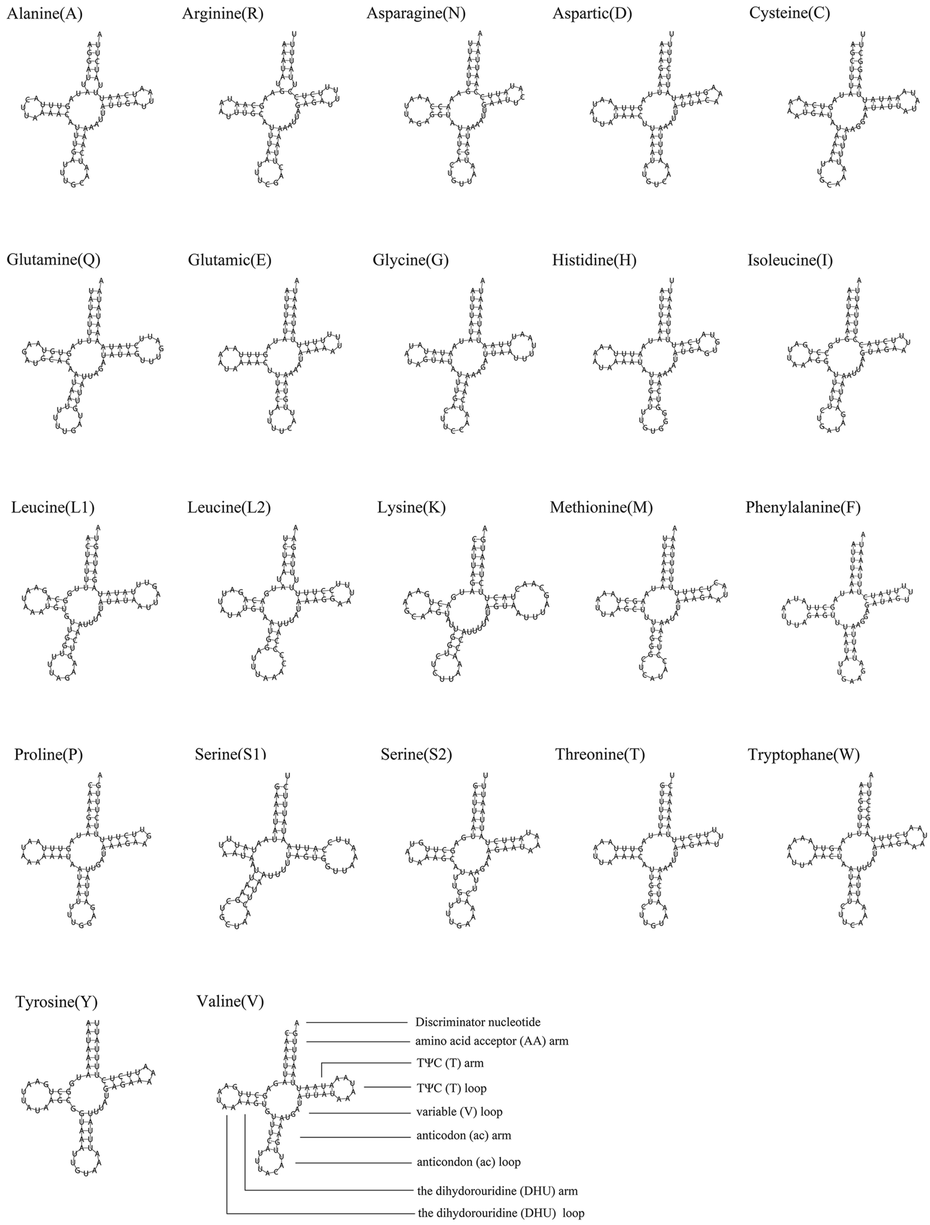

Figure 3. Predicted secondary structure of tRNA genes in the *Triuncina daii* mitogenome. Dashes (-) indicate Watson-Crick base pairing.

The *T. daii* mitochondrial genome contains twenty-two tRNA genes which range from 64 bp to 71 bp in length. Among the 22 tRNA genes, fourteen tRNA genes are mapped on the J-strand and the others are on the N-strand. All tRNA form typical cloverleaf secondary structures (Fig. 3).

Two rRNA genes, *rrnL* and *rrnS*, are 1,392 bp and 863 bp in length, respectively. Both of them were mapped on the N-strand. The *rrnL* is located among *trnL* (CUN) and *trnV* along with the *rrnS* are situated in *trnV* and in the A+T-rich region. The A+T content is 84.48% and 86.21%.

Table 3. Codon usage of the PCGs in *Triuncina daii*

Codon (aa)	N	%	RSCU	Codon (aa)	N	%	RSCU
UUU(F)	366	9.86	1.9	UAU(Y)	178	4.79	1.92
UUC(F)	20	0.54	0.1	UAC(Y)	7	0.19	0.08
UUA(L)	483	12.98	5.24	UAA(*)	0	0	0
UUG(L)	12	0.32	0.13	UAG(*)	0	0	0
CUU(L)	35	0.94	0.38	CAU(H)	59	1.59	1.76
CUC(L)	1	0.03	0.01	CAC(H)	8	0.22	0.24
CUA(L)	22	0.59	0.24	CAA(Q)	60	1.62	1.94
CUG(L)	0	0	0	CAG(Q)	2	0.05	0.06
AUU(I)	441	11.88	1.9	AAU(N)	248	6.68	1.86
AUC(I)	22	0.59	0.1	AAC(N)	19	0.51	0.14
AUA(M)	264	7.11	1.93	AAA(K)	99	2.67	1.87
AUG(M)	10	0.27	0.07	AAG(K)	7	0.19	0.13
GUU(V)	75	2.02	2.14	GAU(D)	59	1.59	1.84
GUC(V)	1	0.03	0.03	GAC(D)	5	0.13	0.16
GUA(V)	60	1.62	1.71	GAA(E)	68	1.83	1.77
GUG(V)	4	0.11	0.11	GAG(E)	9	0.24	0.23
UCU(S)	117	3.15	2.98	UGU(C)	31	0.83	1.94
UCC(S)	15	0.4	0.38	UGC(C)	1	0.03	0.06
UCA(S)	65	1.75	1.66	UGA(W)	92	2.48	1.94
UCG(S)	3	0.08	0.08	UGG(W)	3	0.08	0.06
CCU(P)	69	1.86	2.28	CGU(R)	12	0.32	0.96
CCC(P)	16	0.43	0.53	CGC(R)	1	0.03	0.08
CCA(P)	36	0.97	1.19	CGA(R)	35	0.94	2.8
CCG(P)	0	0	0	CGG(R)	2	0.05	0.16
ACU(T)	81	2.18	2.16	AGU(S)	34	0.92	0.87
ACC(T)	8	0.22	0.21	AGC(S)	0	0	0
ACA(T)	60	1.62	1.6	AGA(S)	80	2.15	2.04
ACG(T)	1	0.03	0.03	AGG(S)	0	0	0
GCU(A)	80	2.15	2.76	GGU(G)	57	1.54	1.19
GCC(A)	4	0.11	0.14	GGC(G)	2	0.05	0.04
GCA(A)	31	0.83	1.07	GGA(G)	112	3.02	2.33
GCG(A)	1	0.03	0.03	GGG(G)	21	0.57	0.44

rrnS—15,122 TATTTAATATAAATTTTTTTACATAGAATTTTTTTTTTTTTTTTTTTATAA
The origin of minority strand replication
TAAAATATTTAATGTAAATTATTAAATTTTTAATATTTCTCTTTCATTTTTTATCTAATA
TTCTAGTTTAAATAAAAAATCAATATTTAAATTTTATAATTCATTTAAATTAAAAAATA
TTATTATAATTAATATTAATTTTTTAATAATTTATTATATTAATATATATATATATATAATA
A microsatellite $(TA)_8$ element
AATAATATAATTTATTATTAATATATTAATTAATTAAAAATTTAATATATATACATATAAA
A microsatellite $(TA)_4$ element
TAAGTAAAATTACTTTTACAAATACCCTAAACCATAATTAATTTTTTTTCATATAAAA
AAAAAAAAA 15,482—*trnM*

Figure 4. Structure of the A + T-rich region of *Triuncina daii*.

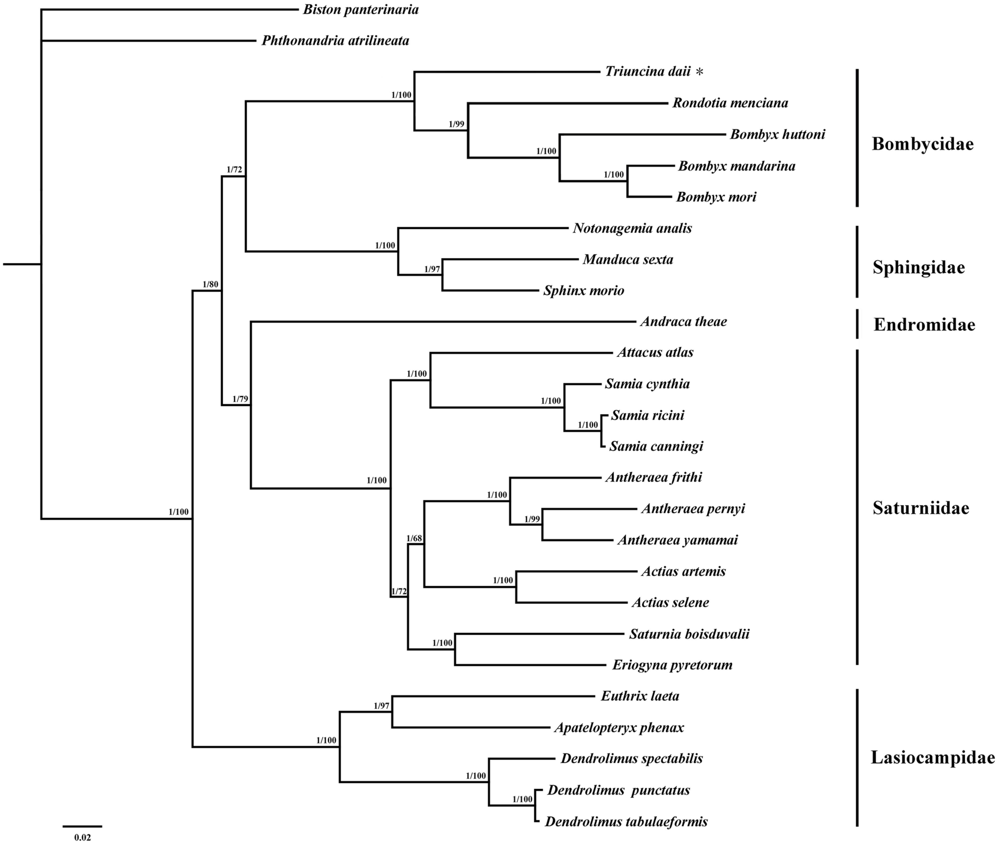

Figure 5. Maximum likelihood and Bayesian inference phylogram constructed using 13 PCGs of mitogenomes with partitioned models. The scale bar indicates the number of substitutions per spot, and the values represent the node supports; above indicates posterior probabilities for the BI and below indicates bootstrap values for ML.

The A+T-rich region of *T. daii*, located between the *trnM* and *rrnS*, is 361 bp in length with a significantly higher A+T content (91.8%). A conserved structure consisting of the motif “ATAGA” and thereafter was 19 bp poly-T stretch. The poly-T is considered to be the origin of the minority strand replication (Fig. 4). Two microsatellites, ‘$(TA)_8$’ and ‘$(TA)_4$’, were found in this region, and they are located 132 and 76 bp upstream of *trnM*, respectively. The poly-A stretch, which is located upstream of *trnM* in other Lepidopteran species, was found and is 13 bp in size.

The phylogenetic relationships within the Bombycoidea, including *T. daii*, were reconstructed and are shown in Fig. 5. The results of both ML and BI analysis revealed that the bombycoids cluster as one monophyletic clade, and the relationships among the Bombycoidea were Lasiocampidae + ((Saturniidae + Endromidae) + (Bombycidae + Sphingidae)), which is strongly supported by a bootstrap value of 100% and a posterior probability of 1.00. While, the other bombycid species were *T. daii* + (*R. menciana* + (*B. huttoni* + (*B. mandarina* + *B. mori*))) with a bootstrap value of 100% and a posterior probability of 1.0.

Discussions

The complete mitochondrial genome of *T. daii* was a closed-circular molecule of 15,482 bp, and the gene order and orientation in the *T. daii* mitogenome is identical to the other lepidopteran mitogenomes (Flook *et al.* 1995) with *trnM*-*trnI*-*trnQ*-*nad2*. The A+T content of nucleotides in the *T. daii* mitogenome was 81.54% which was characteristic of the other bombycid species, such as *Bombyx huttoni* (81.80%), *Bombyx mandarina* (81.68%), *Bombyx mori* (81.32%), *Manduca sexta* (81.79%) and *Sphinx morio* (81.17%) (Table 4). The AT skewness of the *T. daii* mitogenome is 0.003 which indicates a slight bias toward A versus T. The AT content of *trnK* was the lowest, at 70.42%, whereas *trnE* was significantly higher, at 92.31%.

The AT skewness of *T. daii* was higher than *Sphinx morio* (0.001), and lower than *Euthrix laeta, Bombyx huttoni* and *Bombyx mori* (0.019–0.072). However, eleven of 26 mitochondrial genomes of Bombycoidea were T-skewed (Table 4). Wei *et al.* (2010) determined that numerous studies had shown that there was a higher occurrence of T compared with A, and C more than G on the J strand, while in some species it is occasionally reversed. The reason may be related to overcoming background mutation pressures (Meiklejohn *et al.* 2007). Furthermore, Wei *et al.* (2010) stated that the AT skew could be altered by gene direction, replication and codon positions, while the GC skew is not related to gene direction but to replication orientation.

The A+T content in the PCGs was 80.10%, and twelve of 13 PCGs started with “ATN”, while *cox1* started with “CGA” which in lepidopterans may be a synapomorphic feature or diagnostic character (Kim *et al.* 2009). The incomplete stop codon of a single T was found in the *T. daii* mitochondrial *cox1* and *cox2* genes; this phenomenon has been found to be common in several invertebrate mitochondrial genes (Liu *et al.* 2013; Yang *et al.* 2013).

The length of the intergenic spacer regions of *T. daii* (226 bp in 16 regions) range from 1 to 58bp, and the longest non-coding regions are located between *trnF*- *nad5* (58bp). The intergenic spacer between *trnQ*- *nad2* (44bp) was found in the *T. daii* mitogenome, and is conserved among other lepidopteran species (Cameron *et al.* 2008). The overlapping nucleotide fragments are scattered in 6 places, and the spacer between *atp8* and atp6 (7bp) present in *T. daii* is commonly distributed among all Lepidopteran mitogenomes (Jiang *et al.* 2009; Zhu *et al.* 2013; Ma *et al.* 2015). The maximum overlap was between *trnL* (CUN) and *rrnL* (25bp) in *T. daii* while the spacer was not conserved position in other Lepidopteran mitogenomes (Gu *et al*. 2016; Ma *et al*. 2016).

Boore (1999) and Taanman (1999) both thought that the A+T-rich region was the site of gene replication and the initiation of genome transcription (1999). In the *T. daii* mitogenome, the motif "ATAGA" followed by poly-T was found in the A+T-rich region where this motif was a conserved stretch and the poly-T was deemed the origin of the minor strand replication (Saito *et al*. 2005).

Based on the morphology, the taxonomic status of species of the bombycid genus *Triuncina* were uncertain. Strand (1922) transferred the *Triuncina brunnea* to genus *Ocinara* Walker, 1856, and was synonymized with it by Dierl (1978). Moreover, Chu & Wang (1996) incorrectly placed *Triuncina cervina* with *Ocinara brunnea* in the genus *Ocinara*. In recent research, Wang *et al*. (2015) have delineated the species of *Triuncina* to include 5 species in total. Until 2015, the phylogeny of the genus *Triuncina* had not been deeply researched. The present genomic data provides more accurate details than single gene analysis toward understanding the mechanisms of the biological speciation. Since 1990s, numerous molecular phylogenetic studies focusing on Bombycoidea and based on mitochondrial information have been conducted (Kim *et al*. 2000). In our study, the bombycid species were clustered as one clade and *T. daii* is placed in the Bombycidae with a bootstrap value of 100% and a posterior probability of 1.00. The phylogenetic relationships, with strong support, among the Bombycoidea are Lasiocampidae + ((Saturniidae + Endromidae) + (Bombycidae + Sphingidae)) which is consistent with Timmermans et al. (2014) but differs from the findings of other previous studies (Liu *et al*. 2013). To determine the more accurate phylogenetic relationships of the Bombycoidea, more detailed research should be done.

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